# ANIMAL HEALTH AND WELL-BEING

Panel Managers – Dr. Mark Kuhlenschmidt, University of Illinois; Dr. Bradley W. Fenwick, Kansas State University; Dr. Wendy Brown, Washington State University
Program Directors – Dr. Peter J. Johnson, Dr. Peter R. Brayton

The objectives of this program are to increase the knowledge needed to sustain animal health and well-being and to prevent or reduce the severity of animal disease. This includes, but is not limited to: mechanisms that alter the normal physiologic state at the molecular, cellular or organ level to produce disease resulting from either biotic or abiotic causes; cellular mechanisms of disease resistance, including developmental and molecular immunology; microbial genetics/genomics; pathogenesis; both host and microbial factors influencing colonization of mucosal surfaces; host-environment or host-agent interactions that compromise host defense systems or cause predisposition to disease; epidemiologic studies on animal diseases that provide insight into etiologic factors and /or disease control; research that supports the development or evaluation of diagnostic tests and immunizations for emerging or reemerging disease problems such as tuberculosis; studies on economic models that address the costs of animal disease and the cost/benefit ratios of animal disease prevention and therapy. The program also encourages research on the mechanisms controlling animal responses to physical and biological stresses (including quantitative behavioral, physiological, immunological and neurobiological responses to stress) and the development of objective indicators to measure animal well-being.

**2000-02124 Estrogen/Progesterone Modulation of NO Generation in Endotoxemic Calves**
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Grant 2001-35204-10071; $150,000; 3 Years

Lean tissue wasting in catabolic disease is associated with diminished immune function, increased infection rates, decreased wound healing, reduced skeletal muscle function and lowered body weights. All these contribute to reduced animal productivity and well-being. We have proposed that anabolic agents such as estradiol and progesterone (EP) might have a positive impact on an animal's response to disease. Indeed, EP has a remarkable ability to protect calves against coccidiosis and endotoxemia. This project seeks to determine the mechanism for the EP protective effects. We hypothesize that EP inhibits nitric oxide (NO) production. Overproduction of NO has been shown to play a role in the effects of disease on cell damage and cell death. Endotoxemia will be used to model a catabolic disease state and EP treatment examined for its ability to reduce the endotoxin-stimulated generation of the stable end products of NO metabolism (nitrite and nitrate) and modify the activity of the various enzymes regulating NO synthesis. In addition, immune system production of key cytokines will be examined. Based on the results of the first study, the effects of EP will be compared to the effects of specific NO synthesis inhibitors. Determining the mechanisms for EP actions to protect cattle during disease may lead to a new treatment paradigm for use in agriculture and veterinary medicine.

**2000-02772 Molecular and Biochemical Characterization of the *Cryptosporidium* Glycoprotein Ligand CSL**
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Grant 2001-35204-9960; $300,000; 3 Years

Cryptosporidiosis, caused by the ubiquitous parasite *Cryptosporidium parvum*, is a major cause of diarrhea in calves and other agriculturally important food animals throughout the world. The disease also affects humans that are exposed to parasite-contaminated food or water. Treatment and prevention of cryptosporidiosis remain problematic due to the absence of vaccines, and lack of effective parasite-specific drugs. Because cryptosporidiosis is resolved by specific host immune responses, immunologic control strategies have been investigated. To this end, our long term goals have been to identify molecular targets on the parasite, which are recognized by protective immune responses, and to determine their role in the infection process. Limited knowledge on the mechanism of parasite attachment to host intestinal cells has hindered development of vaccines and drug discovery for cryptosporidiosis. Our recent efforts have culminated in the identification of a novel *C. parvum* glycoprotein, designated CSL, which is used as a parasite attachment ligand for intestinal cells. Because CSL is required for attachment to host cells, it is an opportune target for immunologic or pharmacologic intervention against cryptosporidiosis. We hypothesize that parasite attachment to host cells is mediated by functional peptide and carbohydrate domains contained in CSL. The following objectives will test this hypothesis: Objective (1) Identify the glycoprotein domains in native CSL which contain specific attachment molecules for host intestinal cells. Objective (2) Determine if specific attachment of CSL-derived glycopeptides identified in objective 1 is mediated by carbohydrates, or peptides, or both. Objective (3) Determine the complete molecular structure of the CSL glycopeptide-derived components identified in objective 2 which mediate specific attachment required for infection.

**2000-02018 CHANNELING CHICKEN IMMUNITY INTO PROTECTIVE INFLAMMATORY RESPONSE PATHWAYS**

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Strengthening Standard Award; 2001-35204-09957; $160,000; 3 Years

The immune system has two major and separate pathways for fighting off infections. One pathway results in the immune system making large quantities of antibodies and the other in making inflammatory cells that directly fight off the infection. Whether the immune response is dominated by one pathway or the other is often key to surviving infections. Helper T cells which can be either 'Th1' or 'Th2,' determine which pathway is chosen. Very little is known about how Th1 versus Th2 choices are controlled in domestic fowl. We have shifted the response from Th2 to Th1 pathways by macrophage-specific delivery of antigen using chemical modification. These modified proteins (maleylated carrier proteins) bind to scavenger receptors present on macrophages. We will immunize chicks to investigate if the shift can be accomplished in young birds. We will attempt to make a universal bridging protein that can bind to any antigen and also bind to scavenger receptors. We will test that this causes a shift to an inflammatory response. We will modulate immune responses to an infectious agent (Eimeria) to which Th1 immune response is protective. Tests for inflammatory versus antibody-making responses, as well as assays for the ability of T cells to activate macrophages, and express small hormone-like molecules. These data will contribute to better vaccines for poultry diseases.

**2000-02061 Influence of BRSV Infection on Immune Responses to Inhaled Antigens in Bovine Lungs**

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Grant 2001-35204-10070; $270,000, 3 Years

Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory disease in dairy calves and feedlot cattle. Cattle that survive BRSV infection often become unthrifty animals with poor weight gains due to chronic respiratory disease. These cattle negatively impact the economy of the cattle industry. A similar virus that infects human infants, respiratory syncytial virus (RSV), has been shown to enhance development of allergic responses to inhaled allergens. We have data that suggests this may also be true in cattle. This research will elucidate the mechanisms by which BRSV causes allergic enhancement. The experiments focus on a fungal antigen (Aspergillus) present in the feedlot environment as well as a well-characterized egg protein antigen (ovalbumin). We will determine how BRSV infection influences the immune response to these inhaled antigens by studying lymphocytes and molecules in the lymph draining the lung. Immune responses will be correlated with changes in lung function. IgE is the protein made in response to inhaled antigens that is responsible for allergic reactions. The influence of BRSV infection on IgE production will be evaluated by studying IgE production in response to Aspergillus and ovalbumin aerosol in infected compared to uninfected calves. The regulation of the IgE production by cytokines and other factors will be studied in an attempt to determine how BRSV influences the response. Ultimately this project will increase our understanding of the effect of BRSV infection on the immune system and will help us to understand how BRSV infection may participate in chronic respiratory disease.

**2000-02020 Epidemiology of Bovine Viral Diarrhea Virus Infection in Dairy Cows**

Thurmond, M.; Hietala, S.; Johnson, W.
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Grant 98-35204-6390; $200,000; 2 Years

The project will estimate the impact of bovine viral diarrhea virus (BVDV) infection on reproductive performance of cattle. The project will characterize the extent to which the virus affects the subclinical, or inapparent, suboptimal herd performance, such as fertility (eg. days open and services per conception) and fetal survival (eg. abortion) on typically managed commercial dairies. The study is a continuation of a long-term follow-up study of heifers from birth through their second pregnancy (4 years of age) to measure BVDV infection and subsequent reproductive parameters and outcomes. Results will improve our understanding of the effect of BVDV on dairy production efficiency in actual field conditions and, thereby, provide fundamental information about the epidemiology of BVDV as well as a cost-benefit basis for control or eradication. New diagnostic methods will be developed, using pooled-sample testing strategies, aimed at reducing costs and improving efficiency and accuracy of diagnosing BVDV persistent infection, which, although rare, is considered to be the major source of BVDV shedding in herds. These methods also will apply to diagnosis of other rare diseases. To address risk analysis of BVDV on reproductive efficiency, new analytic epidemiologic and statistical methods will be developed. These methods will incorporate Bayesian approaches that will improve our analytic ability to diagnose BVDV-related herd problems, including abortion. The methods also will provide new research and diagnostic tools for general herd-based diagnostics.

**2000-02004 Ninth International Symposium of Veterinary Epidemiology and Economics**
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Grant 00-35204-9201; $9,990; 3 Months

The Ninth symposium of the International Society of Veterinary Epidemiology and Economics (ISVEE) will be held in Breckenridge, Colorado from August 6 to August 11, 2000. The theme of the Symposium will be "The application of epidemiology for the benefit of animal and pubic health". The symposium will feature posters papers, and presentations from many premier animal epidemiologists and a range of topics including animal disease information systems, economic analysis of animal disease and it cost, risk analysis, health constraints to livestock production, delivery of animal health services, and surveillance methods and systems. Six to eight hundred participants from Europe, Africa, Asia and the Americas are expected to attend ISVEE.

**2000-02257 An Evaluation of Strategies to Eradicate Foot-and-Mouth Disease**
Carpenter, T.E.; Thurmond, M.C.; Ardans, A.A.
University of California, Davis; Department of Medicine and Epidemiology School Of Veterinary Medicine; Davis, CA 95616
Grant 2001-35204-9210; $112,000; 2 Year

A disease transmission model will be developed, utilizing data from a hard contact study and existing meteorological resources, as well as expert opinion, to quantify, and visually display the potential size and spatial velocity of hypothetical outbreaks. Alternative eradication strategies, such as different size vaccination or depopulation rings, and depopulation of proximal or otherwise high-risk hers, will be simulated and evaluated for efficacy under multiple epidemic contrary, it will simulate a wide-range of possible epidemic pattern and be used to identify economically-optimal eradication strategies for each pattern. This will enable decision-makers to be prepared prior to an outbreak, or modify their strategy an epidemic has developed. The model will be part of a computer-based decision support system designed to test hypotheses concerning foot and mouth disease (FMD) outbreaks in a well-defined region within California, but could be expanded for nationwide use when additional herd location data becomes available. The model, which incorporates quantitative risk analysis, probabilistic modeling, and economic analysis components, will ultimately be use to address important questions about FMD eradication and be a useful tool for veterinary decision-markers, wen if FMD returns to the United States. Finally, it will be constructed so that it will be relatively easily modified to simulate other diseases or venues.

**2000-02089 Genetic Basis for Antigenic Variation in *Babesia bovis***
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Grant 2001-35204-10144; $300,000; 3 Years

Many pathogenic agents, such as the bovine parasite *Babesia bovis*, establish chronic infections in immune hosts, which can then serve as carriers for transmission of the disease. One mechanism used to evade the host immune system is to rapidly vary certain components, a process called 'antigenic variation', making existing immune responses ineffective. The VESA1

antigen of *B. bovis* undergoes antigenic variation on the infected-erythrocyte surface. Previously, we demonstrated that the *ves*1a gene encoding the VESA1a subunit is a member of a multigene family, with copies on at least 3 of the parasite's 4 chromosomes. We also found preliminary evidence that novel forms of the *ves*1a gene were created by the inclusion of sequences from elsewhere in the genome without obvious modification of the donating sequences- a process called 'gene conversion'. In this project, we propose to critically test the hypothesis that segmental gene conversion, in which many short segments of the gene are modified over time with information from different donor copies, is the primary mechanism used to generate variants.

These information will further our understanding of the mechanisms used by infectious agents to establish chronic infections or to achieve break-through infections in vaccinated animals. This knowledge will benefit U.S. agriculture by enabling us to rationally target the genetic mechanisms that are responsible, thus circumventing this mechanism in a potentially broad range of infectious agents. This could facilitate the development of vaccines or pharacologic agents that target this immunologically- sensitive class of antigens.

**2000-01127 High-Speed Centrifuge for *Actinobacillus pleuropneumoniae* Research and GSU Infrastructure**

Regassa, L.B.
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Equipment Grant; Grant 98-35204-7019; $22,745; 1 Year

This proposal requested funding for a high-speed, refrigerated centrifuge for use in the Biology Department at Georgia Southern University. The centrifuge will be used primarily by the principal investigator to facilitate studies on the swine pathogen *Actinobacillus pleuropneumoniae*. *A. pleuropneumoniae* is the causative agent of porcine pleuropneumonia, a highly contagious and often fatal respiratory tract disease of pigs. Current vaccines are unable to control this costly disease. A better understanding of the mechanisms regulating virulence factor expression may lead to alternative approaches for the treatment of porcine pleuropneumonia. Specifically, the centrifuge will be used to study the production of the Apx toxins, which are important *A. pleuropneumoniae* virulence factors. In addition, two other major research areas involving three additional faculty members in the Biology Department will be strengthened by the presence of the centrifuge. Dr. James Oliver is studying the enzootiology of the Lyme disease spirochete *Borrelia burgdorferi* in the southeastern United States, and Drs. J.B. Claiborne and Alison Morisson-Shetlar investigate how fish utilize their gills to accomplish many of the same tasks that are performed by the kidneys in mammals. These investigators will use the centrifuge for organism recovery and protein isolation. Overall, the acquisition of this high-speed centrifuge will greatly enhance the research infrastructure of the Biology Department at Georgia Southern University.

**2000-02416 Speciation and Transruminal Movement of Tall Fescue Ergot Alklaoids**

Hill, N.S.; Stuedemann, J.A.; Thompson, F.N.; Dawe, D.L.
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Grant 2001-35204-9958; $240,000; 3 Years

Approximately 95% of all tall fescue pastures are infected with a symbiotic fungus. The fungus provides improved agronomic performance to the plant, but adversely affects livestock

consuming the grass because it produces toxins called ergot alkaloids. Twenty-seven percent of the U.S. beef herd is affected by the toxins, costing cattle producers ~ $750 million annually. Little is understood about how the toxins are absorbed, nor how the toxins are metabolized and excreted following absorption. Understanding how the toxins are absorbed and metabolized are pre-requisites to development of strategies to ameliorate the toxicosis effect. The objective of this study is to understand which forms of the toxins are absorbed and at what site they are absorbed within the digestive tract. The objectives will be tested by comparing which alkaloid forms are present in the tall fescue tissue, how they are transformed during the digestive process, and comparing these forms with those in blood and excreted in the urine. To accomplish the objectives we plan to use a combination of *in-vitro* and *in-vivo* techniques. Pastures of endophyte-infected tall fescue will be sampled, and urine collected from grazing cattle. Ruminally digested forage alkaloids will be extracted and quantified after *in-vitro* ruminal fermentation. The forms of ergot alkaloids in grass and digesta will be compared to that in the urine. The alkaloids from digested tall fescue tissue will be extracted and tested for transport across rumen and omasal tissues. Blood samples from gastric blood vessels to confirm absorbed alkaloid forms.

**2000-02069 Black Flies as Epizootic Vectors of Vesicular Stomatitis Virus (New Jersey serotype)**

Mead, D.G.
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Grant 2001-35204-10069; $220,000; 3 Years

Vesicular stomatitis (VS) is caused by a group of antigenically related but distinct viruses of the genus *Vesiculovirus*, family Rhabdoviridae. The disease is of economic importance and primarily affects cattle, swine, and horses, causing vesicular lesions on the mouth, coronary bands, and teats, and less frequently, an influenza-like illness in humans. Other livestock and wildlife species can also be infected. VS has been recognized as a disease of livestock and other animals for over a century, and while the causative agents have been studied intensively in the laboratory, we know relatively little about the viruses as they occur in their natural settings. The maintenance and transmission mechanisms of this economically important group of viruses remain controversial within the scientific and regulatory communities and mixed messages are being sent to those people that VS affects the most -- the livestock owners and producers. A consistent message must be delivered if we hope to initiate economically reasonable, effective, control and prevention protocols that will be followed by the livestock owners and producers. The results of this proposed research will greatly aid this mission. The goals of this proposed research are to determine if VSV-NJ epizootics are dependent on transmission cycles involving black flies, to understand how these vectors become infected with VSV-NJ, to determine if black flies can transmit the virus to livestock species, and to document the clinical response in the animals infected via black fly bite.

**2000-02000 Multidisciplinary Evaluation of Fatal Feedlot Acute Respiratory Distress Syndrome**

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Grant 00-35204-9402; $240,000; 3 Years

Respiratory disease is a leading cause of loss to cattle producers, and acute respiratory distress syndrome (ARDS) is an ongoing cause of respiratory disease in feedlot cattle. Also known as acute interstitial pneumonia (AIP), ARDS often causes death in animals which are growing well and have been on feed greater than 45 days. Significant losses include resources invested in animals that die after a prolonged period on feed. Although ARDS is an important cause of mortality, almost nothing is known about the cause. Bovine respiratory syncytial virus (BRSV) has been implicated, as have bacterial respiratory pathogens and environmental and management factors. Multiple factors may interact simultaneously to cause ARDS. This research will test the hypothesis that BRSV infection superimposed upon low-grade bacterial infection in the lung or gastrointestinal system (liver) is associated with feedlot ARDS. In 3 western U.S. feedlots, tissues from animals with signs of fatal ARDS will be tested for BRSV and bacterial pathogens and compared to controls. This analysis will reveal whether BRSV, bacterial pathogens, or both in combination are significantly associated with fatal ARDS. To determine management or environmental risk factors for ARDS, a prospective case-control study will be carried out. Individual-level factors, such as breed and illness history, and group-level factors, such as ration type and weather changes, will be analyzed for association with ARDS. These studies will identify points amenable to manipulation to control feedlot ARDS, and will form the basis for future studies aimed at decreasing animal disease and decreasing losses to cattle producers.

**2000-02210 Genomic Analysis of *Salmonella* Evolution**

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Grant 00-35204-9196; $343,000; 3 Years

*Salmonella* is a large group of closely related bacteria that are a major cause of food poisoning in humans and animals. The "host-adapted" *Salmonella* have evolved to infect particular animals. However, it is not known how *Salmonella* become adapted to specific hosts, or why some *Salmonella* cause different disease symptoms. To answer these questions, we plan to analyze the DNA sequence of three important host-adapted *Salmonella* to identify the genetic differences responsible for their unique host-adaptation and disease symptoms. *Salmonella* Pullorum, *Salmonella* Dublin, and *Salmonella* Choleraesuis are adapted to different farm animals: *Salmonella* Pullorum infects poultry, *Salmonella* Dublin infects cattle, and *Salmonella* Choleraesuis infects pigs. These three *Salmonella* cause severe, often fatal, diseases in the farm animals. In addition, infected farm animals are a common source of human food poisoning. Each of the serovars shares a core set of common genes, but each serovar also has a set of unique genes that define its distinct virulence properties. To identify these differences, we plan to determine the DNA sequences of the three *Salmonella* genomes, then compare the genome sequences with each other to identify unique genes that may be responsible for their distinct virulence properties. Identifying these genetic differences will provide insight into how these pathogens cause disease, and how pathogens acquire new properties that allow infection of a novel host or alter the disease symptoms. This insight will provide a springboard for the development of new approaches to prevent *Salmonella* infections in farm animals. In addition, because new strains of pathogenic bacteria often arise by infection of a novel host, this insight may help scientists respond to the threat of new, emerging pathogens.

**2000-01997 Demonstrating Key Aspects of Infection and Immunity to *Neospora caninum* in Cattle and Dogs**

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Grant 2001-35204-9961; $200,000; 2 Years

On dairy farms and beef ranches, efficient reproduction is critical to economic success. Abortions (miscarriages) can seriously damage farm productivity. A disease named "neosporosis" (pronounced nee-oh-spore-OH-sis) is one of the most common causes of bovine abortion in the U.S. This disease is caused by a protozoal (one-celled) organism named *Neospora caninum*. The most comprehensive records of neosporosis are available from California, where this disease is estimated to cost the dairy industry $35 million annually. Overall losses to the U.S. cattle industry far exceed this amount because neosporosis is widespread and also commonly affects beef cattle. Recently, research funded by the USDA-NRI revealed that *Neospora* organisms are shed in the feces of dogs after they eat infected animals. This knowledge is a good starting point to begin disease control efforts. However, a blanket recommendation to eliminate farm dogs would be culturally insensitive and would not likely receive widespread acceptance. The current project will seek further information that is needed to control bovine neosporosis. We will confirm that *Neospora* organisms that are shed by dogs are able to cause pregnant cows to abort. Other factors that may affect the outcome of infection will also be investigated, such as the number of organisms that cows must ingest to cause disease, and the infectivity of cattle tissues for dogs. This information will help us to develop practical management recommendations that will help to reduce the incidence of abortion caused by neosporosis.

**2000-02083 Methods for Regionalization and Risk Mapping: Orbivirus Epidemiology as a Model**

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Grant 2001-35204-10153; $300,000; 3 Years

Regionalization is an attempt to partition the risk of infection over spatial and temporal scales. This technique could aid in the minimization of risk of moving infectious pathogens when animals are imported or exported. This process is ideally suited to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV), two pathogens that influence trade restrictions and therefore targeted by many countries for quantitative risk assessments. Bluetongue disease (BT), the clinical manifestation of infection with BTV, is the only Organization International des Epizooties (OIE) Schedule A disease that is endemic in a significant portion of the U.S. Understanding the factors that influence the epidemiology and ecology of these pathogens is critical. However, Culicoides spp., the principal vectors of BTV and EHDV, have not been adequately studied in the midwest U.S., a region that is considered transitional for BTV and EHDV. The factors that determine the annual distribution and abundance of C. *variipennis* complex in the U.S. are uncertain. The epidemiology of EHDV in livestock is relatively unstudied. With all of this uncertainty, the ability to regionalize BTV and EHDV within the U.S. using quantitative and dynamic risk maps is practically impossible. Therefore, the specific

objectives of this project are: 1) Describe the epidemiology of BTV and EHDV within dairy herds in a transitional region of the U.S. 2) Determine the distribution of Culicoides spp. In a transitional region of the U.S. and assess the factors that influence this distribution. 3) Develop predictive models of BTV and EHDV that incorporate host, environmental and vector factors in a transitional region of the U.S., and to apply these models to the development of a dynamic risk map that can be used for regionalization of BTV and EHDV in the U.S.

**2000-02238 Molecular Ecology of the Salmon Gastrointestinal Tract: A Molecular Approach**

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Grant 00-35204-10222; $185,000; 2 Years

Defining the microbial ecology of aquatic gastrointestinal ecosystems facilitates the understanding of microbial relationships pertaining to the animal's health. Gastrointestinal microbial populations have a direct effect on efficiency of animal production. Likewise, the impact of aquaculture on the environment is also effected by the microbial ecology of the aquacultural processes. In the end, the sustainability of the aquaculture industry will benefit from a description of the gastrointestinal microbiota of aquatic food fish. The objectives of this project are to apply an array of molecular ecology techniques based on small submit rRNA (SSU rRNA) sequences to the microbial ecology of healthy and susceptible salmon. Microbial communities of selected hatchery practices (e.g. thermal and dietary regimens) on microbial populations investigated. The molecular approaches employed will result in a complete and accurate description of the microbial community of the gastrointestinal tract, as well as the development of methods for the repaid and accurate detection of microbial density and diversity. This research will result in an increased understanding and more complete description of the gastrointestinal community of food fish under different hatchery conditions and health states, and lead to new strategies for improving food fish health and well-being.

**2000-02197 Defining Novel Diagnostic and Vaccine Targets to Combat Johne's Disease**

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New Investigator Award; 2001-35204-10170; $210,000; 3 Years

Johne's disease is a chronic intestinal disorder particularly common among dairy cattle and it is estimated that 24% of the dairy herds nationally are infected. By some estimates the cost of Johne's disease to dairy farmers exceeds 1.5 billion a year. This disease is caused by *Mycobacterium paratuberculosis*, an intracellular pathogen, whose other family members include *M. tuberculosis* and *M. leprae*, the causative agents of tuberculosis and leprosy respectively. Infected cattle shed high numbers of *M. paratuberculosis* through their feces where it can spread to other members of the herd. A major interest to dairy farmers is the development of diagnostic tools and vaccines for Johne's disease. However, we lack the required information on what *M. paratuberculosis* proteins initiate an immune response in infected cattle and which of these proteins, when used as a vaccine, would protect the calf or cow from Johne's disease. Nevertheless, studies with other pathogenic mycobacteria have identified proteins which produce an immune response in infected animals, including humans. Further, a number of these proteins were shown to protect an animal host from an active mycobacterial disease when used as a

vaccine. We have chosen to clone the *M. paratuberculosis* genes corresponding to these homologous proteins which include GroES, fibronectin attachment protein (FAP), 85abc and the 16-KDa α-crystallin homolog. The proteins corresponding to these genes will be expressed and tested as diagnostic markers to distinguish *M. paratuberculosis* infected from non-infected cattle. The proteins will also be tested as vaccines using a mouse-*M. paratuberculosis* infection model.

**2000-02001 Effects of Prenatal Stress on the Health and Well-Being of Swine**

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Grant 2001-35204-10222; $205,000; 3 Years

The USDA data indicate that swine producers lose greater than 80 million dollars annually from stress induced disease. As with all mammals, swine activate behavioral and physiological systems in response to stress. Although activation of these systems is well recognized, the high degree of variation between animals in their responses to stress is still poorly understood. One important contributing factor to explain this variation is the fact that exposure of a pregnant animal to stress during gestation ("prenatal stress") can result in her offspring being more reactive to stressors in maturity. This exposure to prenatal stress could have a profound impact on the subsequent ability of such offspring to cope with stressful environments and resist disease. Recent studies have demonstrated that stress during fetal development is known to cause many deleterious effects in mature animals, including: increased anxiety and fear behaviors in novel situations, greater secretion and slower clearance of plasma glucocorticoids (known to suppress immune function), feminized sexual behavior of males and masculinized sexual behavior in females, and altered antibody response to disease challenge. While it is clear, therefore, that exposure to prenatal stress can significantly alter an animal's physiology and behavior; we still do not understand the mechanism(s) responsible for this change, or its implications to livestock species. The proposed project will: 1) distinguish which components of the body's "stress system" of prenatally stressed swine are altered and 2) determine the manifestation of prenatal stress on the potential "adaptability" of swine to subsequent stressful or health related events.

**2000-02026 Immunoregulation of Bacterial-Induced Colitis by Porcine T Cells**

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Grant 2001-35204-10183; $180,000; 2 Years

It has been estimated that infectious diseases cost U.S. agriculture in excess of $17 billion annually. A majority of these diseases result from pathogens entering at mucosal surfaces (e.g., lungs, intestinal tract). The overall goal of this project is the development of more effective vaccine strategies for protection against mucosal pathogens. In the proposed study, we hypothesize that T lymphocytes induced by immunization with a proteinase-digested vaccine ameliorate immune-mediated inflammation or hypersensitivity associated with the tissue destruction caused by infection with *Brachyspira hyodysenteriae.* Colitis induced by *B. hyodysenteriae* provides an excellent model to address specific mechanisms of mucosal immunity. We have shown that proteinase-digested bacterins given intramuscularly induce protective immunity with protection correlated with increased numbers of T lymphocytes

expressing the surface marker CD8. Our specific aim is to define the mechanism(s) by which antigen-specific CD8+ T lymphocytes regulate inflammatory and/or immune responsiveness. Specifically, peripheral blood and mucosal lymphocytes will be recovered from vaccinated pigs for phenotypic (by flow cytometry) and functional (i.e., cytokine profiles and proliferative responses) characteristics. In addition, we will stain colonic tissue immunohistochemically to determine in situ changes associated with vaccination and infection. Outbred pigs will be used for both portions of this project. At the conclusion of this project, we expect to have elucidated T lymphocyte-mediated immune mechanisms that regulate host-mediated tissue damage resulting from bacterial-induced colitis in pigs. A better understanding of the peripheral and mucosal immune responses elicited by parenteral vaccination will aid in the practical design of vaccines against other mucosal pathogens.

**2000-02103 Role of Glycoproteins GE and GL in BHV-1 and BHV-5 Differential Neuropthogenesis**

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Grant 00-35204-9534; $285,000; 3 Years

Bovine Herpesvirus 1 (BHV-1), the cause of infectious bovine rhinotracheitis (IBR) and BHV-5, the cause of encephalitis in calves, are neurotropic viruses that establish latency in sensory ganglion. The major clinical difference between these viruses is their ability to cause neurological disease in calves. While both can cause upper respiratory infections, BHV-5 invades the central nervous system. We have established a
rabbit seizure model that distinguishes between acute BHV-1 and BHV-5 nervous system infections.

**2000-00996 Quantification of Feeding and Drinking Behavior of Poultry for Enhanced Animal Well-Being**

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Sabbatical Strengthening Award; Grant 00-35208-9314; $150,709; 1 Year

How individual poultry are affected by heat stress, and what managers can do to offset heat stress, are being investigated in this work. Heat stress for livestock and poultry continues to be a major economic problem faced each summer by producers. It reduces productivity (number of eggs layed, or growth rate) and it causes needless discomfort, loss of appetite and in extreme cases death. Generally, drinking water also becomes very warm during heat stress events. We will determine whether cool drinking water will reduce impact of a hot environment.

A set of 24 individual bird unit (IBU) recording systems have been constructed to provide real-time measurement of how much individual birds eat and drink, and how much time they spend eating and drinking. The IBUs will be placed in four environment-control chambers so that heat stress conditions (like those experienced every summer in the U.S) can be simulated. Experiments are planned to learn whether providing cool drinking water can help offset heat stress, and whether birds that are acclimatized react differently than those who have not been heat stressed previously.

Besides common production parameters (feed and water intake, weight gain, and feed conversion ratios), we shall also categorize individual bird activity from video recordings, measure body core temperature and take blood samples. A key feature of this research is that variation among birds subjected to identical conditions will be directly measured. Reducing heat stress in even a small population may have considerable impact on economics and animal well-being.

**2000-02413 Pathophysiologic and Therapeutic Implications for Endothelin in Equine Laminitis**

Moore, R.M.; Eades S.C.
Louisiana State University; Department of Veterinary Clinical Sciences; Baton Rouge, LA 70803
Grant 2001-35204-10068; $216,481; 2 Years

Laminitis is a debilitating, excruciatingly painful, and often life-threatening or career-ending disease of the sensitive and insensitive laminae of the equine digit (foot). Our global hypothesis is that the initiating factor in the onset of laminitis is a disruption in the release of mediators produced by the cells lining blood vessels (endothelium) in the foot, which leads to a substantial disruption in digital blood flow. We believe an imbalance develops with decreased production of nitric oxide (NO, which normally relaxes blood vessels and increases blood flow) and increased release of endothelin-1 (ET-1, which causes blood vessel contraction and subsequent decreases in blood flow). This ultimately leads to decreased laminar blood flow, laminar swelling and necrosis, and subsequent separation of the sensitive and insensitive laminae resulting in either rotation or distal displacement of the coffin bone within the hoof capsule. This study will investigate alterations in digital vascular variables, digital venous plasma NO and ET-1 concentrations, histologic and immunohistochemical staining of the digital vessels and laminae of horses with laminitis experimentally-induced via carbohydrate overload. These variables will be measured and compared between laminitic horses treated with an endothelin antagonist and those receiving a vehicle-control solution. Knowledge gained from this study may help unravel the events involved in the initiation of laminitis, and thus may help in the prevention and treatment of this devastating disease.

**2000-02213 Further Development and Evaluation of Live Attenuated Vaccines for Channel Catfish**

Thune, R.L.
Louisiana State University; Department of Veterinary Science; Baton Rouge, LA 70803
Grant 00-35204-9195; $280,000; 3 Years

Previous USDA NRICGP support was used to develop and characterize genetically defined, live attenuated vaccine strains of *Edwardseilla ictaluri*, the causative agent of the most important bacterial disease of channel catfish, Enteric Septicemia of Catfish (ESC). Essentially, the live attenuated strains we developed retain their ability to invade catfish from the water, are capable of persisting for up to 72 hours in the host, and elicit excellent protection against ESC in channel catfish. This technology was recently granted US Patent No. 6,010,705, entitled "Attenuated, invasive vaccines against fish pathogens". We now propose to expand the utility of our *E. ictaluri* strain by developing a DNA vaccine delivery system that will provide protection against both ESC and the channel catfish her pesvirus, CCV. Although live attenuated strains of CCV are available and are protective, their production for commercial use requires expensive

cell culture procedures. Although individual CCV disease outbreaks are catastrophic, the unpredictable and sporadic nature of the outbreaks precludes the expense associated with mass vaccination against CCV using currently available methodologies. Our proposal to deliver a DNA vaccine against CCV in our live attenuated ESC vaccine, however, would engender little or no additional cost and would provide significant economic benefit.

**2000-02243 Etiology of Juvenile Oyster Disease of Cultured *Crassostrea virginica***

Boettcher, K.J.; Barber, B.J.
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Strengthening Standard Award; 00-35204-9392; $100,000; 2 Years

The Eastern oyster, *Crassostrea virginica*, is the primary species of bivalve cultured in the northeastern United States. However, since 1988, many producers in this region have experienced annual mortalities of juvenile oysters (up 90% of total production) due to a syndrome of unknown etiology. Juvenile oyster disease (JOD) is now prevalent in several areas in Maine, Massachusetts, and New York, and there is currently no way to predict the impact of JOD or test for its presence. We have accumulated compelling evidence that this is a bacterial disease, and that a novel species of marine bacterium (designated CVSP) is the etiological agent. In this study, we will attempt to reproduce JOD-signs and mortalities in laboratory-maintained *C. virginica* by exposure to CVSP-bacteria. A potential probiotic bacterium (isolated from oysters that were apparently immune to the disease) will also be investigated for its ability to protect juvenile oysters from JOD. Finally, we will investigate how these bacteria become established in oysters, and determine which tissues are affected. Conclusive identification of the JOD-agent will result in detection methods that will provide immediate economic benefits for the industry, and be invaluable for long-term management of the problem. New information about the factors mediating bacterial colonization of the animals will also be forthcoming. As intensive culture of oysters and other bivalves continues to expand in national and economic importance, this research will provide a framework and the tools to better understand, diagnose, and minimize mortalities that result from bacterial infections of cultured shellfish.

**2000-02075 Molecular Basis of Virulence and Persistence in Infectious Pancreatic Necrosis Virus**

Vakharia, V.N.; Baya, A.M.
University of Maryland Biotechnology Institute; Center for Agricultural Biotechnology; College Park, MD 20742-4450

Grant 2001-35204-10065; $200,000; 3 Years

Infectious pancreatic necrosis virus (IPNV) is a pathogen of major economic importance to the nation's 10 billion dollars aquaculture industry. The virus attacks the pancreas of salmonid fish which results in severe morbidity and mortality and the surviving fish become lifelong carriers of IPNV. The goal of our investigation is to identify the viral gene(s) involved in causing this disease. We have developed a system with which one can prepare a "custom-made" virus using the recombinant DNA techniques. Using this system, we plan to prepare chimeras of an avirulent and virulent strains of IPNV by swapping their gene(s). We will then evaluate the properties of these chimeric viruses in fish and identify the gene(s) involved in virulence and persistence. The results of our study will provide the aquaculture industry with critical reagents

for future vaccine developments and minimize losses should highly virulent strains of IPNV emerge in the United States.

**2000-02017 Determination of Sensitivity and Selectivity of a Novel System for Identifying Lameness in Dairy Cattle**

Tasch, U.; Lefcourt, A.M.; Erez, B.; Dyer, R.M.; Varner, M.A.
University of Maryland, Baltimore Campus; Department of Mechanical Engineering; Baltimore, MD 21228

Grant 2001-35204-10118; $147,000; 2 Years

Lameness caused by hoof and leg ailments causes significant economic losses to the dairy industry and is an animal welfare issue of great concern, The goal of this project is to develop a system that detects lame cows and identifies the troubled limbs. Moore specifically, we would like to identify a set of limb movement characteristics that are indicative of hoof and leg ailments at their early onset before the cow can be identified as lame by visual observation, Early detection of hoof and leg problems will enable medical interventions that can reduce economic losses, lessen animal suffering, and expedite animal recovery. Early detection will also enable researcher to study and compare the efficacy of various medical treatments and management alternatives. A walkthrough Reaction Force Detection (RFD) device was developed over the last three year. This device has been used to evaluate various limb movement characteristics of sound and lame cows. The PI's propose to conduct an experiment in which the limb-movement characteristics of thirty clinically diagnosed, yet treatable, lame cows are followed from the time of diagnosis through the recovery period. A function that is reliable and sensitive in identifying lameness at the early onset of the ailment is sought. Successful completion of this research will pave the way for the development of an automated lameness diagnostic system that will enhance farm automation and improve animal well being.

**2000-02247 Role of *Cryptosporidium parvum* Surface Glycoproteins in Host-Parasite Interactions**

Ward, H.D.
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Grant 2001-35204-10172; $300,000; 3 Years

*Cryptosporidium* is an intestinal parasite that commonly infects animals of agricultural importance such as calves, lambs, goat kids, piglets, foals and poultry and is a major cause of diarrheal disease in new born animals. In addition, environmental contamination by infected animals can lead to transmission of the parasite to humans resulting in severe illness in immunocompromised individuals such as AIDS patients. There is currently no effective vaccine or treatment approved for this disease. The overall goal of this project is to further our understanding of how this parasite infects intestinal cells, in order to develop methods to prevent and treat the disease. This proposal is focused on determining how two proteins, gp40 and gp15, which are present on the surface of the parasite enable *Cryptosporidium* to infect cells in the intestine. The long-term goal is to determine if methods can be developed to prevent the parasite from infecting intestinal cells by blocking the action of these proteins. A major goal is to determine if these proteins or the DNA which encodes them can be used to develop vaccines or drugs which can prevent or treat cryptosporidial infection in humans as well as in animals such

as newborn calves, lambs and kids. Developing vaccines or drugs for cryptosporidial infection would be of great importance in contributing to the health and well-being of animals of agricultural importance, in reducing the economic impact of the disease and in preventing environmental contamination with this parasite and transmission to humans or other animals.

**2000-02293 Memory Response of Bovine WC1+ Gamma Delta T Cells**

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Grant 00-35204-9222; $247,000; 2 Years

As new infectious diseases emerge and antibiotic resistant strains of bacteria develop, the need for new vaccines increases. It would also be advantageous to have methods to stimulate the immune system in a global manner to prevent infections that occur due to suppression of the immune system resulting from stress (such as for shipping fever) and to combat infections caused by unknown agents. The research in this proposal concerns a unique type of cell in the immune system known as a gamma delta T lymphocyte (or T cell). They are white blood cells that comprise the majority of T cells in ruminants during the first few months after birth and remain at high levels in the blood for the first several years of life. Thus it is reasonable to presume they are important components of the immune system of cattle. It is known that they play a role in limiting viral and bacterial infections. Our goal is to understand the role of these cells in protective immunity in ruminants. "Memory" is a hallmark of responses by the other major type of T lymphocytes known as alpha beta T cells and B lymphocytes that produce antibodies. Their ability to "remember" is the keystone of vaccination. The studies proposed here will help us determine whether gamma delta T cells undergo physiological changes akin to establishment of memory cells. If so, vaccines that stimulate these cells may be generated to prevent or alleviate infectious disease and increase animal health and well-being.

**2000-02215 Genome Sequencing and Analysis of Mycobacterium Paratuberculosis**

Kapur, V.; Bannantine, J.; Stable, J.; Bolin, C.
University of Minnesota, St. Paul; Department of Veterinary Pathobiology; St. Paul MN 55108; and USDA Agricultural Research Service; National Animal Disease Center; Ames, IA 50010

Grant 00-35204-9200; $575,000; 2 Years

Mycobacterium Paratuberculosis cause Johne’s disease in cattle and other small ruminants. This disease has a major economic and animal health impact in the United State and other parts of the world. Despite the tremendous loss associated with M. paratuberculosis infections, we lack effective diagnostic teste and vaccines for preventing the disease. This is in large part due to a lack of basic understanding of the bacterium and the molecular basis for its ability to infect animals and cause disease. We have proposed a collaborative project to determine the complete nucleotide sequence of this bacterium. To accomplish this objective, we will utilize a random shotgun sequencing strategy that as been successfully applied in the past for whole genome sequence analysis of microbial and other organisms. The results of the investigation will help in the identification of all of the genes responsible for microbial replication, virulence, species-specificity, and ability to evade the immune system. We believe that this comprehensive knowledge of the pathogen’s genome will provide all the necessary information required for cost-effective and targeted

research into disease prevention and treatment such as the development of superior diagnostic tests and vaccines.

**2000-02307 Characterization and Role of *Pasteurella haemolytica* Leukotoxin Receptors**
Maheswaran, S.K.; Kannan, M.S.; Walcheck, B.K.
University of Minnesota; Department of Veterinary PathoBiology; St. Paul, MN 55108
Grant 00-35204-9230; $216,000; 3 Years

Bovine pneumonic pasteurellosis (BPP) commonly known as shipping fever remains a leading source of economic loss to the beef and dairy cattle industries in the U.S. through treatment costs, death of affected animals, and reduced production performance of those which survive. The estimated annual loss exceeding $1 billion and the loss of potential food constitutes an intolerable waste of the U.S. resources. Evidence suggests that the lung injury in BPP is caused by inflammatory substances released by lung white blood cells as a result of the interaction of leukotoxin produced by the causative bacterium, *Pasteurella haemolytica*. Current treatment protocols emphasize the use of injectable antibiotics to eliminate the bacteria, but therapy is frequently unsuccessful because it does not reverse the inflammation in the lungs, once initiated. Furthermore, growing consumer anxieties regarding antibiotic residues in meat and dairy products, as well as the emergence of multi-antibiotic resistant bacteria make industry dependence on antibiotics undesirable. How can we prevent the acute lung injury in shipping fever? We hypothesize that therapeutic approaches designed to block leukotoxin interaction with its receptors in lung white blood cells will undoubtedly prevent the release of various inflammatory substances and prevent subsequent lung injury. The objectives of this research project are to identify and characterize the leukotoxin receptors in the lung white blood cells of cattle, and determine how the binding of the leukotoxin to its receptors results in the release of inflammatory substances. We will use basic approaches to address these objectives. Our goal is to use this understanding to develop new methods of prevention and treatment that will suppress inflammatory damage in the lungs of cattle affected with shipping fever.

**2000-01131 A Gel Documentation System for Use in Agricultural Research**
Coats, K.S.
Mississippi State University; Department of Biological Sciences; Mississippi State, MS 39762
Equipment Award; Grant 2001-35208-9860; $19,557; 1 Year

The gel documentation system will be used in a variety of agriculture-related projects by numerous investigators. The equipment will provide a means to document, annotate, and quantitatively evaluate images of proteins and nucleic acids on gels and in bacterial colonies. The following projects will examine mechanisms of disease and/or vaccine development in agriculturally-important animals. All projects will benefit from this equipment: (1) An evaluation of virological and immunological parameters that predispose maternal-fetal transmission of bovine immunodeficiency virus in dairy cattle will be done. A competitive PCR assay will be used to measure viral RNA in maternal and fetal circulations and to quantitate expression of placental immunomodulators. (2) Mycoplasma gallisepticum causes chronic respiratory syndrome in poultry, resulting in egg production losses. Live vaccines control infections, but produce pathology in chickens. The development of an improved, recombinant vaccine, incorporating the adhesin genes of vaccine strains of Mycoplasma gallisepticum is the goal of this project. The gel documentation system will be used to identify candidate adhesin genes. (3)

The polysaccharide capsule of Pasteurella multocida, the etiologic agent of avian cholera, is an important virulence factor of this bacterium. A lipoprotein (Plp-40) is thought to play a role in encapsulation of this bacterium. Expression of the protein in encapsulated and unencapsulated bacteria will be examined by radiolabeling the proteins and quantifying them using the gel documentation system. (4) Quantification of channel catfish virus (CCV) replication and virus mRNA transcription will be done using the gel documentation system as a part of ongoing research to optimize the development of a recombinant CCV vaccine vector. (5) Evaluation of catfish genes associated with resistance to intracellular pathogens such as Edwardsiella ictaluri will be done. The gel documentation system will allow quantitation of mRNA associated with resistance genes.

**2000-01106 Identification of Neurotoxins from Centaurea: A Systematic Approach**
Rimoldi, J.M.; Matthews, J.C.; Slattery, M.
University of Mississippi; Department of Medicinal Chemistry; University, MS 38677
Seed Grant; Grant 2001-35208-9969; $75,000; 2 Years

The prolonged feeding of yellow starthistle or Russian knapweed by horses has been linked to a fatal neurodegenerative disorder termed equine nigropallidal encephalomalacia (ENE). Both of these weeds can become the major green foliage in a horse's diet; and affects small land owners who cannot move their horses to other pastures not infested with the weed. Commonly known as "chewing disease", ENE causes severe impairment in eating and drinking, aimless walking or drowsy inactivity, and ultimately, death. A plant toxin is believed to be the agent responsible for ENE; however, experimental evidence confirming the connection between this plant toxin and ENE onset has not been firmly established. Our proposed research is aimed at establishing whether this plant toxin, or, a fungal pathogen growing on the weeds, contributes to this fatal disorder. We propose to systematically evaluate both the organic- and water-soluble weed extracts by utilizing bioassay-guided fractionation using a neuronal cell line, and to compare seasonal differences in the weed ecology employing gas chromatographic "fingerprinting". Previous studies have indicated a strong seasonal correlation of ENE (typically highest during the wet winter season), and there is some evidence that certain weed populations have higher levels of the toxic components or fungal pathogens. Our study of the weed ecology will provide additional information requisite to understanding the etiology of ENE, ultimately leading to the control of this devastating equine disease.

**2000-02267 Regulation of IgA Responses in Cattle**
Estes, D.M.
University of Missouri; Department of Veterinary Pathobiology; Columbia, MO 65211
Grant 2001-35204-10066; $255,000; 3 Years

A major outcome of vaccination and host responses to infectious agents is the development of an antibody or humoral immune response. Antibodies, a class of proteins present in body fluids or tissues, have an important role in preventing disease. Neutralizing antibodies can block entry of pathogens into tissues. As the airways and intestinal tract are the major portals of entry for most pathogens, antibodies present at these sites can be a deterrent to development of disease. This series of studies will evaluate the mechanisms that control production of antibody types which predominate at these sites. Ultimately, these studies will provide a basis for improving host immunity in the lung/upper airways and gastrointestinal tract.

Respiratory and GI tract diseases of cattle are a major source of economic loss for both the beef and dairy industries. The knowledge gained by completion of these studies will facilitate the development of improved first and second generation vaccines for a variety of economically important cattle pathogens.

**2000-02222 Genetic Analysis of Avian *E. coli* Virulence**
Curtiss III, R.
Washington University; Department of Biology; St. Louis, MO 63130-4899
Grant 00-35204-9224; $270,000, 3 Years

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of airsacculitis, septicemia, pericarditis and perihepatitis in poultry. The use of production intensive confinement housing has resulted in the emergence of this organism as one the predominant bacterial diseases affecting the poultry industry. Specific strains of *E. coli* are associated with a number of human and animal diseases, and those of serotype 01, 02 and 078 predominate in avian colibacillosis. Recent studies have shown that unique DNA regions on the genome of pathogenic *E. coli* strains are associated with virulence in a particular niche. Our long-term objective is to establish the genetic basis for the ability of avian pathogenic E. coli to colonize the respiratory tract and cause systemic disease. Our specific objective for the grant period are to: (1) identify unique genes that are expressed by APEC in vivo in infected chickens, (2) construct derivatives with mutations these genes and flanking regions and determine if they are important for virulence, and (3) identify by complementation cloning and transpose mutagenesis individual genes that contribute to virulence. In subsequent years, we will evaluate the biochemical mechanisms by which significant virulence factors function and establish the means by which their expression is regulated. The research will contribute to the basic knowledge towards the mechanisms by which *E. coli* cause disease in poultry, provide sequences to use as diagnostic and epidemiologic tools, and provide information useful for the constructions of vaccines to prevent APEC infection in poultry. The research will make use of genetic, biochemical and animal science techniques.

**2000-01114 *Tritrichomonas Foetus*: Genotypes and Virulence**
Burgess, D. E.
Montana State University, Bozeman; Veterinary Molecular Biology; Bozeman, MT 59717
Seed Grant; Grant 00-35208-9241; $73,286; 2 Years

Trichomoniasis occurs in beef cattle, particularly range cattle. Trichomoniasis causes economic losses including first trimester abortions. This project examines the relationship of parasite virulence to parasite genes. The purpose of this project is to identify DNA sequences that may control parasite virulence.

**2000-01238 *Mycobacterium paratuberculosis* Survival in Biofilms**
Hall-Stoodley, L.; Veeh, R.
Montana State University, Bozeman; Center for Biofilm Engineering; Bozeman, MT 59717
Seed Grant; Grant 00-35208-9192; $75,000; 2 Years

*Mycobacterium paratuberculosis* causes paratuberculosis or Johne's disease in wild and domestic ruminants. Paratuberculosis is responsible for significant economic losses in the US, particularly in dairy herds. Once detected, elimination of the organism in a herd is problematical and may take several years. Although infection occurs via the fecal-oral route, little is known

about the ability of this organism to survive in the environment and its resistance to control efforts. Mycobacteria aggregate and form clumps, which may influence their ability to survive environmental stresses. It is now recognized that many types of bacteria aggregate on surfaces to form biofilms in the environment. Attached bacterial communities appear to better survive deficient growth conditions, temperature fluctuations and chemical treatment. Recent evidence also shows that other species of mycobacteria form biofilms. *M. paratuberculosis* within biofilms could serve as a potential reservoir of Johne's disease and facilitate persistence even after eradication efforts have been implemented. By investigating the biofilm forming capacity of *M. paratuberculosis*, we will examine a possibly novel mode of this pathogen's survival. This research will provide information toward a better understanding of the survival of *M. paratuberculosis* in the environment (including water supplies), improved detection of the organism, and possibly superior methods for controlling its spread.

**2000-01200 Equipment Grant for an Ultracentrifuge Rotor Package**

Hardy, M.E.; Pascual, D.W.; Schmidt, E.E.

Montana State University; Veterinary Molecular Biology; Bozeman, MT 59717

Equipment Grant; Grant 2001-35208-9869; $24,750; 1 Year

Veterinary Molecular Biology at Montana State University has developed an integrated research program in animal health and development. Many research efforts in the department are focused on understanding mechanisms of gene regulation in pathogens and host cells. The ability to control expression of specific genes underlies the complexity of pathogen replication, of cellular responses to infections, in mechanisms of host defense, and in development of eukaryotic cells. Gene expression is regulated at multiple levels including RNA and protein synthesis, and downstream regulation by interactions with other gene products. These pathways must be understood in order to develop technologies that result in improved animal health and fitness. Ongoing projects include: (1) investigation of viral gene expression and analyses of specific viral protein interactions with host cell proteins, (2) delivery of antigens to mucosal sites to stimulate effective immune responses, (3) biochemical role of the leukocyte oxidant-generating systems in host defense, and (4) utilization of transgenic models to understand regulation of spermatid developmental pathways that are important in infertility. This equipment grant is for purchase of a Beckman swinging bucket rotor package for preparative and analytical ultracentrifugation. SW28, SW41Ti, and SW55Ti rotors are essential tools for techniques such as virus purification, protein purification, and analyses of protein: protein interactions. Due to rotor de-rating and obsolescence, our current departmental capabilities in ultracentrifugation are inadequate to support ongoing and future research projects. The rotors will enhance our productivity by providing essential equipment necessary to meet the technological needs of our research programs.

**2000-02115 Regulation of Rotavirus Gene Expression - Mechanisms of Translational Control**

Hardy, M.E.

Montana State University; Veterinary Molecular Biology; Bozeman, MT 59717

Grant 2001-35204-10072; $220,000; 2 Years

Rotaviruses are a leading cause of diarrhea in newborn calves. Rotavirus infections also can increase the susceptibility of calves to secondary or concurrent infections with other

pathogens such as E. coli, Salmonella, and Cryptosporidium. New intervention strategies need to be developed that will inhibit rotavirus replication in the host, maintain a high standard of animal health, and reduce the economic burden of diarrheal diseases on the cattle industry. In order to design rational strategies for development of replication inhibitors, the basic functions of the viral proteins that are necessary for virus replication must be understood at the molecular level. These studies focus on understanding the roles of nonstructural protein NSP1 and the untranslated regions in viral mRNA in regulating viral protein synthesis. We have formulated the hypothesis that NSP1 regulates rotavirus gene expression at the level of protein synthesis by specific interactions with viral mRNA and cellular proteins in infected cells. To address this hypothesis, we will perform experiments to identify nucleotide sequences in the untranslated regions of viral mRNA that regulate viral protein synthesis, and dissect how these sequences participate in controlling the level of proteins encoded by the 11 rotavirus genes. We will determine how NSP1 interacts with these sequences, and perform functional assays to understand how the molecular interactions regulate protein expression or viral RNA transport. Our long-term goal is to develop novel therapeutics for bovine rotavirus based on inhibition of virus-encoded replication functions. Such inhibitors should be low cost, easily administered, and amenable to large-scale use.

**2000-02012 Development of Yeast Systems to Study Toxoplasma Cell Cycle**

Kvaal, C.A.
Montana State University, Bozeman; Department of Veterinary Molecular Biology; Bozeman, MT 59717

Post Doctoral Fellowship; Grant 2001-35204-9959; $90,000; 2 Years

Many apicomplexan parasite diseases of livestock are untreatable, and where treatments exist, the inventory of drugs is often limited and aging, while the number of drug resistant parasites continues to rise. The U.S. livestock industry incurs hundreds of millions of dollars in treatment costs and production losses each year fighting a losing battle against these parasites. There are consequences beyond agriculture, as many protozoan pathogens of livestock and their relatives are threats to human populations. Recent outbreaks of coccidial disease caused by Cryptosporidium in water (1993 in Milwaukee, WI >400,000 people infected), Cyclospora contaminating fruits (1996, 1997 Calif. >1,000 people infected), and Toxoplasma in water (1995 in Victoria, Canada up to 10,000 individuals infected), amply demonstrate that these pathogens can strike populations at any time without warning. The goal of this proposal is the identification of genes controlling the unusual cell cycle of picomplexan parasites. We have chosen Toxoplasma gondii as a model system to explore picomplexan cell cycle regulation at the molecular level. We will exploit the power of yeast genetics as a surrogate experimental system in two of three specific aims, searching for the effector proteins of cell cycle control in *T. gondii*. Given the atypical nature of apicomplexan parasites cell cycle, discovery of cell cycle control proteins of T. gondii will provide new targets for drug development in apicomplexa.

**2000-01138 Equipment Grant for a Thermal Cycler with Gradient and *In Situ* Polymerase Chain Reaction Capability**

Quinn, M.T.
Montana State University; Department of Veterinary Molecular Biology; Bozeman, MT 59717

Equipment Grant; Grant 00-35208-9186; $12,485; 1 Year

An essential requirement for the conduct of cutting-edge research in cellular and molecular biology is the availability of modern research instrumentation. For example, ultra-sensitive new techniques have recently been developed for studying the pathology of infectious diseases in livestock. One of the most sensitive of these techniques is in situ polymerase chain reaction (PCR). In situ PCR is performed directly on non-disrupted cells and tissue sections and is the only hybridization technique that allows cellular and subcellular localization of the target DNA sequences. Consequently, it provides a very powerful tool for answering many questions that could not have been otherwise investigated. Currently, there are no thermal cyclers with these capabilities in this department and only one older instrument capable of this technique at our entire university. Thus, the goal of this equipment grant is to obtain a state-of-the-art thermal cycler with gradient and in situ PCR capability in the Department of Veterinary Molecular Biology at Montana State University. This instrument will allow us to fully develop in situ techniques for use in analyzing host defense and infectious diseases in livestock, thereby enabling us to utilize this highly sensitive technique in a wide variety of ongoing projects in veterinary molecular pathology. Clearly, the availability of a thermal cycler with gradient and in situ capabilities would expand and expedite our current studies by adding new research capabilities and enhance these projects' competitiveness in future grant applications.

**2000-02262 Regulation of the Neutrophil NADPH Oxidase in Bovine Blood and Milk**

Quinn, M.T.
Montana State University; Department of Veterinary Molecular Biology; Bozeman, MT 59717
Grant 2001-35204-9956; $150,000; 2 Years

White blood cells called neutrophils play an essential role in protecting the udder against bacterial pathogens. These cells respond to the presence of a pathogen by migrating to its location, engulfing the pathogen, and generating microbicidal oxidants, which kills the pathogen. The importance of neutrophils in defending the udder is demonstrated in bovine mastitis, which results from infection of the mammary gland and is characterized by a rapid influx of many neutrophils into the infected quadrant(s). This influx of neutrophils into the udder reduces the yield and quality of the milk; thereby, causing a significant economic impact on the dairy industry. On the other hand, neutrophil function is essential for defense against mastitis, and a correlation between decreased neutrophil function and severity of mastitis has been demonstrated. We propose to utilize reagents recently developed to address questions regarding how one key microbicidal system (NADPH oxidase) is regulated at the molecular level in both blood and milk neutrophils. To accomplish this goal, we will analyze assembly and activity of the NADPH oxidase in bovine blood and milk neutrophils treated with various activating agents and then analyze the expression of NADPH oxidase proteins at the protein and nucleic acid levels in resting and activated neutrophils from bovine blood and from milk of normal and mastitic animals. The results of these studies will increase our understanding of the regulation of the bovine neutrophil NADPH oxidase system and could eventually lead to the development of strategies for modulating the inflammatory aspects of bovine mastitis.

**2000-02031 Thioflavine T Detection of the Prion Protein**

Bartz, J.C.
Creighton University; Department of Medical Microbiology and Immunology; Omaha, NE 68178

Postdoctoral Fellowship; Grant 00-35204-9228; $89,858; 2 Years

Identification of transmissible spongiform encephalopathy (TSE) strains has taken on increased importance with the emergence of bovine spongiform enchephalopathy in the United Kingdom and its subsequent transmission to other species including humans. Chronic wasting disease is an emerging TSE of deer and elk in the United States that has an unknown host range and may pose a risk to sheep and cattle since they share pasturelands. Currently, no method exists to monitor for the prevalent or emerging TSE strains of livestock and wild animals. The objective of this proposal is to develop a rapid method for surveillance of TSE strains in livestock. An assay will be developed to identify TSE strains based on the ability of a fluorescent dye to interact with a TSE-specific protein in a strain-specific manner. This will be accomplished by first, optimizing the reaction conditions. Second, the number of distinct strains that can be identified in a single host species and in how many host species can the same TSE strain can be identified will be investigated. Finally, isolates of naturally occurring TSEs from the U.S. will be examined in order to examine TSE strain diversity in livestock. The ability to monitor endemic and emerging TSE strains in domestic animals can facilitate control measures to reduce the spread of TSEs and to assess the risk that emerging strains pose to animal and human health.

**2000-02198 Role of Macrophages in the Pathogenesis of Porcine Colonic Spirochetosis**

Duhamel, G.E.; Cirillo, J.D.
University of Nebraska, Lincoln; Department of Veterinary and Biomedical Sciences; Lincoln, NE 68583-0905

Grant 00-35204-9313; $240,000; 3 Years

*Brachyspira pilosicoli* is a spirochete bacterium that causes colonic spirochetosis, a newly emerging intestinal disease affecting human and non-human primates, pigs, dogs, and various species of domestic, wild and zoo birds. Without considering the costs of medication, the decreased weight gain and diarrhea caused by colonic spirochetosis may cost the U.S. swine industry as much as $244.8 million per year. Macrophages are one of the body's key defenses against infection of the intestine by virtue of their ability to engulf and degrade bacterial pathogens. Investigations into the interaction of *B. pilosicoli* with macrophages revealed that, following uptake by a new mechanism called coiling phagocytosis, the spirochetes persisted and replicated within infected macrophages. We proposed that *B. pilosicoli* survives within the macrophages because it modifies the normal pathways of bacterial traffic within the cell. The aim of the project is to examine the mechanisms of uptake and intracellular trafficking and survival of *B. pilosicoli* within porcine macrophages. The results of these studies will provide a better understanding of the ways *B. pilosicoli* interacts with macrophages and will help develop a broad understanding of mechanisms involved in other bacterial infections. Our long-term goal is to develop new approaches for treatment and prevention of bacterial intestinal infections of humans and animals.

**2000-02060 Inhibition of Apoptosis by the Bovine Herpes Virus 1 Latency Related Gene**

Jones, C.J.; Doster, A.
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Grant 00-35204-9262; $292,000; 3 Years

Bovine Herpesvirus 1 (BHV-1) is a significant viral pathogen of cattle that can cause upper respiratory disease, abortions, "shipping fever", or occasionally encephalitis. After acute infection, the primary site for BHV-1 latency is sensory neurons located in trigeminal ganglia. Virus can persist in a latent state for the lifetime of the infected host or periodically reactivate. The ability of BHV-1 to establish a latent infection and reactivate from latency is the main reason why BHV-1 is maintained in the field. Latency also complicates designing effective vaccines or using modified live vaccines in the cattle industry. During a latent infection, viral gene expression is limited to a single latency related (LR) gene. The ability of LR gene products to prevent programmed cell death (apoptosis) is believed to promote neuronal survival when BHV-1 infects neurons. The focus of this study is to construct a mutant strain of BHV-1 that does not express LR gene products and compare the pathogenic potential of this mutant to wildtype virus in cattle. The parameters that will be studied include virus shedding during acute infection, establishment of latency, reactivation from latency, and apoptosis in trigeminal ganglia. Additional studies will map sequences in the LR gene that are necessary for inhibiting apoptosis and determine the cellular genes that play a role in inhibiting apoptosis. Understanding the function of the LR gene may allow us to design vaccine strains that do not undergo latency and reactivation.

**2000-02268 Gp96 as a Molecular Chaperone for Antigen Delivery in Viral Systems**

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Grant 00-35204-9331; $200,000; 2 Years

Bovine herpesvirus 1 (BHV-1) is an important etiological agent of bovine respiratory disease complex which costs over $500 million annually to US cattle industry. Like wild-type virus, modified live virus (MLV) vaccine strains of BHV-1 also induce immunosuppression. Epitope-based vaccines may offer a better alternative to currently used MLV vaccines. Epitopes are short amino acid sequences on viral proteins that induce cytotoxic T-lymphocytes (CTLs) and antibodies specific for the virus. Success of epitope-based vaccines depends on their ability to direct CTL epitopes to the major histocompatibility complex (MHC) class I antigen presentation pathway. Endoplasmic reticulum-resident heat shock protein (hsp) gp96 associates with a wide array of peptides which are not selected by the MHC haplotype of the harboring cell. Hence gp96-peptides can be used to induce virus-specific CTLs and antibodies without prior identification of the epitopes, and across the MHC barrier. In preliminary studies in our laboratory, immunization of mice with hsp-peptide complexes, from transfectants expressing BHV-1 glycoprotein gD (BC-gD), resulted in BHV-1-specific CTLs and antibodies. Therefore, the first objective of this project is to extend our finding by showing that gp96 isolated from BC-gD elicits BHV-1 gD-specific CTLs and antibodies in cattle. The second objective is to determine the protective efficacy of immunization of calves with gp96-peptide complexes from BC-gD by challenging the immunized calves with virulent BHV-1. Success in this BHV-1 model should lead to the development of efficacious vaccines for other important viral pathogens as well.

**2000-02212 Can a DNA Vaccine Induce Cutaneous Immunity in Fish?**

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Grant 00-35204-9223; $280,000; 3 Years

DNA vaccines have recently emerged as important new tools in the fight against infectious disease. Such vaccines are composed of naked DNA molecules which can be expressed as foreign protein following their introduction into recipient animals. In mammals, resulting immune responses have been shown to provide strong protection against a variety of microbial pathogens. Recent studies have suggested that DNA vaccines may be useful in aquaculture as well. To test this idea, attempts will be made to develop a DNA vaccine against *Ichthyophthirius multifiliis*, a protozoan parasite of freshwater fish. As the etiologic agent of "white spot" disease, *Ichthyophthirius* has substantial impact on commercial aquaculture in this country and abroad. In addition, it provides a unique model for the study of host-parasite interactions leading to immunity in fish. We have identified antigens on the parasite surface which can elicit protective resistance against *I. multifiliis* and have cloned the genes encoding these antigens. These genes are being modified for expression in teleosts, and administered by different routes to channel catfish and rainbow trout as recombinant DNA vaccines. Immune responses are being tested by measuring serum and mucus antibody levels in vaccinated fish, along with protection following direct parasite challenge. Aquaculture represents one of the fastest growing areas of agribusiness in the United States. Because fish raised under intensive farming conditions are highly susceptible to infectious disease, the use of DNA vaccines may prove extremely useful in boosting productivity within this arena.

**2000-02066 Marek's Disease Virus-induced Cytokine and Nitric Oxide Production *in vivo***
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Postdoctoral Fellowship; Grant 2001-35204-10152; $89,926; 2 Years

Marek's disease (MD) is characterized by the development of tumors in chickens that are infected with a herpesvirus called Marek's disease virus (MDV). When infected with certain strains of MDV, chickens may develop tumors in multiple organs leading to death, and condemnation of chickens used for food. It was estimated that in 1984, monetary losses due to MD totaled about $12 million. Chickens are vaccinated against MDV and it has been estimated that condemnation due to MD in broilers would reach 1 billion chickens if it were not for these vaccines. However, recently extremely virulent MDV strains are appearing, thereby making the available vaccines less efficient against these viruses. Little is known about the non-specific and specific immune responses during a MDV infection and we propose to study certain non-specific immune responses. One aspect is the expression of cytokines, which are molecules produced by immune cells that direct the immune system to react to the virus. In addition, nitric oxide can not only modify the responses of the immune system but can also affect MDV replication. We expect that the expression of these molecules over the course of an infection may ultimately determine whether a chicken will develop tumors. Knowledge of the specific molecules expressed during an infection will undoubtedly lead to the development of better vaccines, which would hopefully protect chickens against the newly emerging virulent viruses.

**2000-02092 Mechanisms of Intestinal Repair in Infectious Enteritis**
Argenzio,R.A.; Rhoads, J.M.
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Grant 2001-35204-10207; $250,000; 2 Years

The long term goals of these laboratories are to better understand the cellular mechanisms of diarrhea and tissue injury and to define mechanisms of intestinal mucosal defense and repair in infectious enteritis. After mucosal injury of the small intestine, rapid restoration of epithelial continuity and normal barrier permeability depends on a process termed restitution, in which epithelial cell migration rapidly covers the denuded basement membrane. Our preliminary studies with acutely injured porcine intestinal mucosa showed that the amino acid arginine plus serum maximally stimulated this process while either alone was without effect. Furthermore, these products synergistically stimulated migration in a cell culture model; an effect blocked by a nitric oxide synthase inhibitor. Our central hypothesis proposes that the direct effects of arginine and serum are mediated by nitric oxide and transforming growth factor beta, which increase cell migration via separate downstream signaling pathways. Furthermore we propose that this stimulated migration is intact in *cryptosporidium*-infected tissue and would thus provide a practical means to promote intestinal repair in infectious enteritis. We will study functional and morphological epithelial repair in control and infected piglet ileum acutely injured in Ussing chambers, and treated with arginine and or serum in the presence or absence of antioxidants and nitric oxide synthase inhibitors. In a cell culture system, we will determine which growth factors in serum stimulate migration and identify the intracellular signal transduction pathways activated by arginine and serum by immune complex assay.

**2000-01199 Pulsed-Field Gel Electrophoresis to Enhance Research of Animal Pathogens**
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Equipment Grant; Grant 98-35204-7018; $23,000; 1 Year

A pulsed-field gel electrophoresis (PFGE) sytsem, such as the Bio-Rad CEF Mapper XA system (Hercules, CA), is a powerful tool to separate DNA over 50 kb in size and has numerous applications that will enhance research into animal health problems and their impact on food safety. Specifically, a CHEF Mapper will enable us to perform PFGE-based experiments to characterize and strain-type bacterial pathogens isolated from animals and from food products. In particular, it will serve our research into the virulence and antimicrobial resistance mechanisms of microbial pathogens and methods to control these microorganisms. We have a collection of about 500 avian E. coli isolates. 400 of these were isolated from birds wit E. coli infections including septicemia and cellulitis. Almost 100 were isolated from healthy birds. These isolates have been well characterized as to the possession of virulence factors. These isolates been serotyped and phage typed, and it was found that there were more than 50 serotypes represented among this with group with about 40% being non-typeable by traditional techniques. PFGE will permit us to strain-type these isolates in a more coherent way. We will also be able to determine the genomic location of the *increased serum survival* gene, which may be a marker of virulent avian *E. coli*. PFGE will be a valuable epidemiologic tool to investigate disease outbreaks in animal populations as well as food-borne pathogens. Additionally, PFGE will expedite the process to confirm genomic mutations. In sum, the addition of a PFGE system to our campus should profoundly improve the research infrastructure of our department, our competitiveness for extramural funds, and ability to collaborate with other animal health researchers.

**2000-02427 *Pasteurella haemolytica* Lipopolysaccharide Leukotoxin Complex**

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Grant 00-35204-9312; $174,000; 2 Years

Shipping fever pneumonia in cattle is a multi-factorial disease initiated by environmental stress and viral infection which predisposes affected cattle to bacterial infection by Pasteurella haemolytica A1. Only reproductive failure and neonatal enteric disease cause losses of a similar order of magnitude to shipping fever. Despite progress made in combating this disease, shipping fever pneumonia continues to cost U.S. beef producers and consumers >$800,000,000 annually. Husbandry practices limit the effectiveness of vaccinal or antibiotic control of shipping fever. This proposal seeks to provide basic information necessary to develop strategies to reduce the effectiveness of two of the most potent P. haemolytica virulence factors, leukotoxin (LKT) and lipopolysaccharide (LPS). The hypothesis for this proposal is that: the structural association of LPS and LKT facilitates interaction of LPS with host immune cells causing synergism in the aberrantly increased production of proteins which cause excessive and unproductive inflammation. The specific objectives are: 1) To compare host cell production of inflammatory products elicited by LPS-LKT complex with that for LPS-free LKT, isolated LPS, and non-complexed LKT + LPS to determine whether LKT facilitates LPS immune cell activation, and 2) To identify receptors mediating LKT facilitated LPS immune cell activation. This information may lead to development of non-antibiotic, but prophylactic treatment aimed at reducing the effectiveness of P. haemolytica virulence factors in establishing infection.

**2000-02209 Genetic Construction of Bovine CD18 that Resists *Pasteurella haemolytica* Leukotoxin**

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Grant 00-35204-9227; $150,000; 2 Years

*Pasteurella (Mannheimia) haemolytica* is the cause of an economically important respiratory disease of cattle known as “shipping fever”. The purpose of this project is to investigate the relationship between a toxin (leukotoxin, LKT) secreted by the *Pasteurella* bacterium and bovine immune cells. The main objective of this project is to definitively identify the bovine cell receptor that LKT uses to bind and mediate toxicity. Following identification of the receptor, the specific binding domain will be characterized. The information generated from this work will lead to improved treatment regimens, vaccine strategies, and possibly the development of cattle genetically resistant to “shipping fever”.

**2000-02106 Uses of Baculovirus Vectors for the Development of Fish Vaccines**

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Grant 2001-35204-10116; $200,000, 3 Years

If current trends continue, by the year 2025 people will consume more than 55 million tons of seafood annually. It is clear that harvesting the ocean's fisheries to meet these needs is not sustainable and thus, the bulk of those 55 million tons will have to come from aquaculture. Our long-range goal is to develop commercially viable vaccines for disease control in fish

hatcheries. One of the critical factors that must be considered in the development of vaccines for fish is the requirement for efficient delivery of vaccines to large numbers of fish at one time. The infectious hematopoietic necrosis virus (IHNV) is a serious pathogen of salmonid fishes, especially when reared under hatchery conditions. A DNA vaccine that gives effective protection against IHNV when injected into rainbow trout fry has been developed in Dr. Leong's laboratory. However, injection vaccination is not practical on a large scale, and a more efficient means of delivery of this vaccine needs to be developed, such as oral or immersion administration. We have found that insect baculoviruses can be exploited for the delivery of genes to cultured fish cells, and thus have potential for the delivery of DNA vaccines to fish in vivo. Our strategy is to determine a set of optimized conditions for baculovirus delivery of genes to fish, and use this knowledge to design baculovirus vectors and vaccination protocols that can be used to protect rainbow trout against IHNV disease.

**2000-02123 Haemonchus Contortus: Neuronal Control of the Infective Process**
Schad, G.A.; Gamble, H.R.; Ashton, F.T.
University of Pennsylvania; Department of Pathobiology; Philadelphia, PA 19104
Grant 2001-35204-10154; $300,000; 3 Years

This project seeks to discover and identify the specific chemosensory neurons located in the anterior sensory organs (known as amphids), that indicate the arrival of the stomach worm *Haemonchus contortus* larva in its host, and these initiate processes that cause resumption of development of the larva. Identification of these neurons will provide a basis for understanding the infective process. This will provide information that should facilitate the discovery and development of preventative strategies for the control of stomach worm infection (and other nematode infections) in domesticated ruminants. We have mapped the cell bodies of the amphidial nerves, and assigned names to the individually recognized neurons. Using differential interference contrast microscopy and laser microsurgery, we have identified the main thermosensory neurons. In the planned new studies, infective larvae in which specific amphidial neurons, or sets of these neurons, have been ablated will be tested in an in vitro culture system for their ability both to recognize host-like conditions and to respond by resuming development. We anticipate that, in contrast to controls, operated larvae in which the neurons that recognize presence in the host have been ablated will fail to recognize simulated host-provided signals and will fail to develop. Experiments of similar design will identify the sensory neurons used by incoming larvae to recognize and respond to tissue- and organ-specific signals mediating site selection. In vitro results will be tested in jirds to provide in vivo confirmation (or contradiction) of specific neuronal control of infective processes.

**2000-02506 Role of Complement in the Antibody Response of Teleost Fish**
Sunyer, J.O.
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Grant 00-35204-9221; $250,000; 3 Years

Aquaculture is the fastest growing animal food sector in the U.S and is poised to become a major U.S. growth industry in the 21$^{st}$ century. Disease and health management problems are one of the major hurdles for the developing aquaculture industry in the US , which could thwart or prevent the continued development of the industry. Without research and development of the aquatic animal health sector in the US these businesses may fail to thrive. Disease and health

management issues are still largely unresolved because of our lack of knowledge of many basic aspects of the immune system in fish. One of the areas that require further attention is our understanding of the immune mechanisms that lead to the development of an antibody response in fish. Better knowledge in this area is fundamental to the development of new and improved therapeutic tools, including vaccines, as well as new strategies for fish immunization. In this project we propose to assess the role of complement in mediating and influencing the antibody response in fish. In mammals, the complement system plays an important role in protecting the organism from viruses, bacteria and other microorganisms and also plays a major role in modulating the antibody response. Our long term goals are to further our understanding of the antibody response in fish, including mechanisms of antigen uptake, internalization, processing, and presentation, and to evaluate the role of fish complement in each of these processes. The results of our proposed experiments may have important implications for the future use of complement as a natural adjuvant in newly developed complement-based vaccine preparations for fish.

**2000-01264 Feed-based Delivery of Recombinant Antimicrobial Peptides for Shellfish Aquaculture**

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Seed Grant; Grant 00-35208-9315; $71,355; 2 Years

Microbial pathogens and parasites place a large economic burden on aquaculture. The research described in this proposal offers an alternative approach to disease prevention and treatment by building upon an innate mechanism of disease resistance in a wide variety of species, the production of antimicrobial peptides (AMPs). The long-term goal of our research is to develop a method for the feed-based delivery of AMPs to finfish and shellfish. The goal of the research proposed here is to determine the feasibility of feed-based delivery of AMPs to oysters in a study with the following specific aims: (1) Screen for candidate AMPs that are effective against marine pathogens and can be expressed in eukaryotic hosts; and (2) optimize the conditions for expression of AMPs in well-characterized yeast expression systems. The proposed strategy takes advantage of the wide knowledge on yeast expression systems for optimization of the conditions for production and delivery of AMPs. While the current work will focus on testing the methodology in the widely used yeast expression system, our preliminary results indicate that the strategy could be adapted in the future to microalgal expression systems. This novel strategy offers the potential benefits of reducing pathogen burden in the water system in aquaculture, as well as boosting endogenous levels of specific AMPs within the animal tissues to either prevent or treat pathogen outbreaks. These microalgal expression systems have the potential for widespread applications in aquaculture.

**2000-01163 Refrigerated Superspeed Centrifuge and Rotors for Basic Laboratory Research**

Nelson, D.R.
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Equipment Grant; Grant 98-35204-7017; $22,200; 1 Year

This project is to purchase a refrigerated superspeed centrifuge (Sorvall RC-5C and for

centrifuge rotors (SS-34, SLA-1500, SLA-3000, and HB-4). The centrifuge will be used to carry out basic laboratory procedures including harvesting of bacterial cells, preparation of subcellular fractions, preparation of various experimental materials, such as, Atlantic salmon gastrointestinal mucus and mucus extracts. The centrifuge will replace a 30+ year old Sorvall RC-2B refrigerated superspeed centrifuge and rotors (each 17+ years old). The current centrifuge has become inoperative due to age and the unavailability of replacement parts. The current rotors are old enough to be derated or retired. This equipment will support the research of the investigator, which includes one USDA/NRI funded investigation (Mucus-inducible genes and proteins of Vibrio anguillarum as vaccine candidates)), one NIH-funded investigation (Role of tick saliva in Lyme disease and vaccine strategy), and one investigation funded by Alpharma, Inc. a pharmaceutical company and fish vaccine producer (Development of safe and effective methods for the immunization of fish using DNA).

**2000-02114 Genomic Quasispecies Associated with the Persistence and Pathogenesis of Porcine Reproductive and Respiratory Syndrome Virus**

Benfield, D.A.; Rowland, R.R.
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Grant 2001-35204-10171; $200,000; 2 Years

Porcine reproductive and respiratory syndrome is the most economically important viral disease affecting the U.S. swine industry. The porcine reproductive and respiratory syndrome virus (PRRSV) causes acute and persistent infections in pigs of all ages. The major impediment to control of this disease by either vaccination or management protocols is the ability of PRRSV to temporarily persist in pigs. Persistently infected pigs are considered the main epidemiological source of virus within and between herds. Generally, the number of pigs persistently infected within the first two months after exposure is 100 percent. While the number of persistently infected pigs declines over time, virus and viral nucleic acid can still be detected in a few pigs up to 251 days after infection. Unfortunately, the mechanism via PRRSV persists in animals is not known, but tonsils are the best tissue for recovery of PRRSV from persistently infected pigs. One hypothesis to explain persistence is that PRRSV undergoes selection or mutation to adapt to the changing environment within the pig as the disease progresses from an acute to persistent infection. The immune response and/or changes in tissue tropism may drive the selection of PRRSV that either evade the immune response or compartmentalize in specific tissues most suitable for long- term replication. The goal of experiments in this proposal is to determine the temporal and spatial diversity of the PRRSV as the virus progresses from an acute to a persistent infection. Information on how these viral variants or quasispecies evolve may explain how this virus persists in pigs and evades elimination by the immune response.

**2000-02200 Biochemical Basis for Genetic Resistance to K88 *Escherichia coli* Infections**

Erickson, A.K.
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Grant 99-35204-8689; $160,000; 3 Years

Diarrhea (scours) caused by the bacterium, enterotoxigenic *Escherichia coli* expressing the K88ac fimbrial adhesin (K88ac ETEC), is a significant source of economic loss to the pork production industry in the United States. The long-term goal of my research is to identify new

approaches to prevent and treat scours caused by K88ac ETEC. These approaches will be based on a better understanding of attachment of K88ac ETEC to the cells that line the inside of the piglet's small intestine. Previous studies have indicated that some pigs are genetically susceptible to K88ac ETEC infections, while others are resistant to infection by K88ac ETEC. Susceptible pigs are thought to express attachment sites on their intestinal cells for the bacteria to bind to, while resistant pigs lack these attachment sites. Our hypothesis is that susceptible pigs express a unique carbohydrate structure on their intestinal cells that serves as the attachment site for K88ac ETEC. This site is absent in pigs that are resistant to K88ac ETEC infections. To test this hypothesis, we will identify the carbohydrate that serves as the attachment site for K88ac ETEC and determine the structure of this carbohydrate. This information will then be used to devise strategies for efficiently identifying resistant pigs. These resistant pigs can then be used in breeding programs to eliminate K88ac ETEC susceptible pigs from the swine population.

**2000-02202 The Pasteurella (Mannheimia) haemolytica A1 Genome Sequence**
Highlander, S.K.; Weinstock, G.M.
Baylor College of Medicine; Department of Molecular Virology and Microbiology and Human Genome Sequencing Center; Houston, TX 77030
Grant 00-35204-9229; $353,000; 2 Years

*Pasteurella (Mannheimia) haemolytica* A1 is the primary bacterial pathogen involved bovine respiratory disease, or shipping fever. This disease causes losses of up to $1,000,000,000 to the US cattle industry each year. Effective vaccines to prevent bovine respiratory disease have yet to be developed. To gain new insight into the virulence mechanisms of this important pathogen, the complete DNA sequence of the chromosome of strain PHL213 (ca 2,700,000 base pairs) will be determined to an accuracy of 99.9%. Sequences will be determined by automated DNA sequencing of randomly selected cloned short (2-4 kilobase) and long (20-30 kilobase) fragments of the chromosome. Large insert clones will be used to prepare a 100 clone "scaffold" on which to array the smaller random sequences. DNA sequences will be assembled and potential genes and gene products identified by using a variety of bioinformatics programs and databases. Assembled and annotated sequences will be made freely available to the scientific community by placing the information on a microbial genomes web page at the Baylor College of Medicine Human Genome Sequencing Center. DNA clones and plasmids will also be made available for distribution.

**2000-02305 Role of Major Soluble Antigen in Pathogenicity of Bacterial Kidney Disease**
Rhodes, L.D.; Strom, M.S.
DOC National Marine Fisheries Service; Northwest Fisheries Science Center; Seattle, WA 98112
Grant 00-35204-9225; $141,000; 2 Years

Approximately 20% of the seafood consumed in the U.S. is farm-raised, and domestically farmed salmon averages 33 million pounds and $76 million annually. In addition to direct food production, salmon are reared for stocking and conservation efforts. Consequently, diseases affecting cultured salmon have implications for farmed and released salmon. Bacterial kidney disease is a debilitating condition that is transmitted horizontally (in water) and vertically (in eggs). While infections are typically chronic, stress such as crowding or smoltification can produce acute disease and widespread mortalities. How the causative bacterium, *Renibacterium salmoninarum*, subverts the salmon’s immune defenses is unknown. A surface protein of *R.*

*salmoninarum*, major soluble antigen (MSA), has been identified and cloned. The abundance of MSA protein and the presence of two identical copies (rather than a single copy) of the msa gene suggest the general hypothesis that MSA is important for cellular interactions, such as with host immune cells. To test this hypothesis, a variant of *R. salmoninarum* that expresses very low levels of MSA will be used for comparisons with wild type (which expresses high levels of MSA). Differences in interactions with macrophages, site of entry into fish, and ability to provide protective immunity by vaccination will be evaluated between the variant and wild type lines. A null mutant (no MSA) will be created by disruption of the MSA gene for future testing. If MSA is shown to be essential for causing BKD, the null mutant may be the basis of a live vaccine.

**2000-02076 IHN Virus Traffic Between Farmed, Hatchery, and Wild Salmonid Fishes**
Garver, K.A.
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Postdoctoral Fellowship; Grant 00-35204-9226; $90,000; 2 Years

Aquaculture is a growing industry that is making increasingly important contribution to the food supply of the United States. One of the most productive aquaculture areas within the contiguous United States is the Columbia River basin. Hosting both an anadromous salmonid hatchery production system and a large trout farming industry, salmonid aquaculture within the Columbia River basin supports a $52 million dollar per year industry . However, the productivity of aquaculture operations within the Columbia River basin is often limited by disease outbreaks in both fish farms and hatchery facilities. One of the most significant diseases within the Columbia River basin is caused by infectious hematopoietic necrosis (IHN) virus. IHN virus causes frequent outbreaks in numerous locations within the Columbia River basin every year and accounts for major economic losses. Additionally there is concern that such outbreaks of IHN virus in aquaculture facilities could impact the health of cultured and wild salmonids throughout other areas of the Columbia River basin. However, at present there is no scientific data to determine if this is a significant problem. Therefore, the intent of this proposal is to investigate IHN virus samples from hatchery and wild fish throughout the entire Columbia River basin to assess whether there is IHN virus transmission between farmed, hatchery, and wild salmon populations. By conducting both field and laboratory studies, we will enhance our understanding of virus traffic patterns throughout this major watershed.

**2000-02087 Lymphocyte-Mediated Immunity in Cattle during Acute Infection with *Anaplasma marginale***
McGuire, T.C.; Valdez, R.A.
Washington State University; Department of Veterinary Microbiology and Pathology; Pullman, WA 99164 Grant 2001-35204-10117; $171,434; 2 Years

Anaplasmosis is one of the most prevalent tick-borne diseases that continue to constrain the production of cattle worldwide. In the U.S., estimates of losses range from $30 to $60 million dollars per year. Despite extensive losses, immunization against the causative rickettsial pathogen, *Anaplasma marginale*, with a safe and effective vaccine is not yet feasible on a practical basis. Complete development of an effective anaplasmosis vaccine has been inhibited by the lack of knowledge of basic mechanisms of natural immunity required for protection. The goal of the proposed research is sequential dissection of *in vivo* mechanisms of protective

immunity during acute anaplasmosis. The hypothesis that $CD4^+$ T lymphocytes and the cytokine IFN-γ are required in cattle for control of acute infection of cattle with *A. marginale* will be tested directly in the ruminant host. The strategy is to deplete thymectomized calves of $CD4^+$ T lymphocytes, using an anti-bovine CD4 monoclonal antibody, followed by experimental infection of calves with *A. marginale*. Parameters of diseases will be compared between depleted and non-depleted calves. If $CD4^+$ T lymphocytes are necessary and sufficient for control of acute anaplasmosis, then the hypothesis that this control is mediated by the cytokine IFN-γ will be tested. IFN-γ will be neutralized *in vivo* by administration of an anti-bovine IFN-γ monoclonal antibody followed by experimental infection of calves with *A. marginale* and comparison of disease parameters between treated and non-treated calves.

**2000-02226 Epithelial Cell Invasion by *Edwardsiella ictaluri***
Skirpstunas, R.T.
Washington State University; Department of Veterinary Microbiology and Pathology; Pullman, WA 99164-7040
Postdoctoral Award; Grant 00-35204-9211; $90,000; 2 Years

This research will investigate initial interactions between channel catfish epithelial cells and *Edwardsiella ictaluri*, the bacterium that causes Enteric Septicemia of Catfish (ESC). Initial research will focus on development of a model *in vitro* system to study bacterial - host cell interactions, since such a model is currently unavailable. Utilizing this model, specific molecules on the bacterial surface used to gain entry into susceptible host cells will be identified. Proteins located on the surface of the bacterium will be isolated and antibodies against these proteins will be generated. These antibodies will be used as reagents to block bacterial entry into host cells using the model (*in vitro*) system, and thus corresponding proteins important in initial interactions between the bacterium and host cell will be identified. Identification of these molecules will facilitate development of more efficient vaccines, critical for control of this devastating disease of farmed catfish.

**2000-02295 Induction of Diagnostically Valuable *M. paratuberculosis* Antigens**
Collins, M.T.
University of Wisconsin, Madison; Department of Pathobiological Sciences; Madison, WI 53706
Grant 00-35204-9311; $199,000; 3 Years

Johne's disease affects over 22% of U.S. dairy herds. This intestinal bacterial infection costs the dairy industry millions annually in decreased milk production, lower carcass weight, veterinary expenses and higher herd turnover. Blood tests for Johne's disease lack sensitivity. They detect less than half of the truly infected cattle in a herd. One possible explanation for this inadequate test performance is that the test is missing key ingredients. These key ingredients are bacterial elements (antigens) derived from the organism that causes Johne's disease (*M. paratuberculosis*). In theory the test contains the same bacterial antigens as encountered by the infected animal. However, since test antigens are harvested from bacteria produced under optimized laboratory conditions designed to encourage growth, not under the harsher and varied conditions experienced within the animal, it is likely that the antigens used in the test do not actually mimic the antigens seen by the animal. This project will test the theory that growing *M. paratuberculosis* under conditions that closely mimic those found inside animal cells will produce different bacterial antigens. It is expected that these different antigens will be useful in

developing better diagnostic tests for this costly agricultural disease.

**2000-02102 Bovine Interleukin-1 Receptors and Interleukin-1 Receptor Accessory Protein in Mastitis**

Czuprynski, C.J.
University of Wisconsin, Madison; Department of Pathobiological Sciences; Madison, WI 53706
Grant 2001-35204-10067; $100,000; 2 Years

Interleukin-1 is a small protein that is released by white blood cells in response to microbes and microbial toxins. Interleukin-1 is a key player in coordinating inflammation, and the immune response. Regulation of the biological activity of interleukin-1 is complex. The interleukin-1 family consists of three ligands, two receptors and at least one accessory protein. Nearly all the research on the interleukin-1 family has been done using rodent or human cells. There has been little investigation of interleukin-1 in cattle or other food animal species. Mastitis is an economically significant disease problem in which interleukin-1 likely plays an important role. In the proposed research, we will investigate the regulation of bovine interleukin-1 ligands, receptors, and accessory protein in vitro, in response to bacteria and bacterial toxins, and in vivo during bacterial mastitis. There are two specific objectives: (1) Compare bovine peripheral blood granulocytes, mononuclear cells, and mammary epithelial cells, for their expression of interleukin-1 ligands, receptors, and accessory proteins in response to the pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus in vitro*; and (2) Evaluate the expression of interleukin-1 ligands, receptors, and accessory proteins *in vivo* using experimental coliform and *S. aureus* mastitis as models for acute and chronic mastitis, respectively. Accomplishing these objectives will increase our understanding of how the bovine interleukin-1 family is regulated, and may provide new insights into ways by which interleukin-1 might be manipulated to enhance host defense.

**2000-02304 Role of Purinergic Receptors in Endothelial Cell Damage by *Haemophilus Somnus***

Czuprynski, C.J.
University of Wisconsin, Madison; Department of Pathobiological Sciences; Madison, WI 53706
Grant 00-35204-9212; $180,000; 2 Years

*Haemophilus somnus* is a versatile bacterial pathogen that is of considerable economic importance to the U.S. beef and dairy cattle industries. *H. somnus* infection frequently involves inflammation and damage to blood vessels (ie. vasculitis and thrombosis) that are lined with endothelial cells. The long term goal of the proposed research is to understand the mechanisms by which *Haemophilus somnus* causes damage to endothelial cells, and how this relates to the blood vessel damage that is seen *in vivo*. The central hypothesis we will test is that bovine endothelial cells undergo programmed cell death (i.e. apoptosis) when *Haemophilus somnus* adheres to or invades them, or when these cells are incubated with a component of the outer membrane of the bacteria (lipooligosaccharide). A second hypothesis is that a certain type of receptor on the endothelial cell surface (purinergic receptors) mediate, at least in part, the adverse affects of *Haemophilus somnus* and its lipooligosaccharide on bovine endothelial cells. These evaluations will be done using bovine endothelial cell lines and various isolates of *Haemophilus somnus.* Cell death will be evaluated by a number of molecular and morphological assays. The role of purinergic receptors will be evaluated using various inhibitors of these receptors. We also

may examine tissues from some cattle with *H. somnus* infection for signs of apoptotic endothelial cells. The information obtained in this study might illuminate potential new targets for intervention in efforts to reduce the severity of disease in *Haemophilus somnus* infected cattle.

**2000-02048 Generation of Cytotoxic Lymphocytes and Immunity to Equine Herpes Virus by DNA Vaccination**

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Grant 00-35204-9265; $273,000; 3 Years

The objective of this proposal is to develop a DNA vaccine that will protect horses from equine herpesvirus (EHV) infection, one of the most important infectious diseases affecting the horse throughout the world. We have developed the technique of DNA vaccination for use in horses. This radical new innovation in vaccination technology uses directly injected viral DNA to induce immune responses. We have already successfully used DNA vaccination to protect horses from influenza virus infection. We are now ideally positioned to develop an EHV DNA vaccine to protect horses from EHV infection, and to determine the role of different components of the equine immune responses in this protection. Scientists currently believe that immune cells called cytotoxic lymphocytes (CTL) play the pivotal role in protecting horses from both primary and latent EHV infections. DNA vaccines are an extremely potent means of generating CTL responses. Our studies will identify the genetic components of EHV-1 that ultimately generate CTL responses, and then use these genetic sequences in DNA vaccination experiments in horses. On completion of this project, we will have identified what types of equine immune response can protect against EHV infection, an essential step in developing any form of EHV vaccine for horses. In addition the EHV DNA vaccine that we will develop may prove to be the first effective vaccine against EHV infection. Overall this project will achieve critical advances towards our long-term goal of developing a multivalent equine DNA vaccine against both influenza virus and EHV infection in horses.

**2000-02053 Pathogenesis and Evolution of H3n2 Swine Influenza Viruses**

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Grant 2001-35204-10184; $184,000; 2 Years

Infection of pigs with influenza viruses is an economically important cause of respiratory disease and, because influenza viruses from pigs can be transmitted to humans, a public health concern. In March 1998, unique H3N2 subtype influenza viruses emerged among U.S. pigs. These strains were derived by genetic reassortment between human, swine and avian viruses. Since they are antigenically distinct from the H1N1 viruses that have caused swine influenza since the 1930s, these H3N2 viruses have been able to spread throughout the country, causing severe (even fatal) respiratory disease in pigs and possibly abortions. In the first part of our work, we will develop monoclonal antibodies (using DNA vaccine technology) and polyclonal antisera to these H3N2 viruses, and we will determine the most efficient method for isolation of influenza viruses from clinical specimens from pigs. We will then obtain viruses to study from a slaughterhouse-based surveillance program and from samples submitted by colleagues from around the country. Using the antibodies that we develop, we will determine how these viruses

change antigenically over time, and we will also study their evolution at the genetic level. This information is critically important for rational vaccine development efforts. In the second part of our work, we will begin to investigate the genetic basis for why some H3N2 viruses readily infect pigs and/or spread efficiently from pig-to-pig, whereas others do not. For this work, we will employ a powerful new, plasmid-based "reverse genetics" system that allows one to create viruses with specific genetic mutations/components.

**2000-01253 Oma87, a Model Protein for a Novel Group of Bacterial Outer Membrane Proteins**

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Seed Grant; Grant 00-35208-9190; $74,999; 2 Years

The Outer Membrane Antigen (Oma87) of *Pasteurella multocida* belongs to a new family of bacterial outer membrane proteins (OMPs). Although no function has yet been assigned to these OMPs, evidence suggests that these proteins are involved in the export of virulence factors that aid the pathogen in the progress of disease. Proteins similar to Oma87 have been found in other bacterial pathogens such as *Brucella abortus*, *Salmonella typhimurium* and *Escherichia coli*. We propose to use Oma87 from a fowl cholera causing strain of *P. multocida* as a model to identify the function of Oma87-like proteins and to elucidate their role in bacterial pathogenesis. Diseases caused by *P. multocida* such as fowl cholera atrophic rhinitis and pneumonia in cattle impact directly on the agricultural industry. These diseases cause significant losses in animals as well as reduce the animals' health. Results obtained from this proposal will strengthen USDA-NRI's ability to achieve its Animal Health objectives which include improving animal health and assuring high quality meat products for the consumer. In addition, because Oma87-like proteins are found in a number of important bacterial pathogens of the agricultural industry, these data will contribute to the basic knowledge of how bacteria interact with their animal hosts and cause disease. Finally, results from this proposal will be used to support future applied research focused on bacterial pathogenesis and the development of effective non-antibiotic control strategies against pathogens that will ultimately improve animal health.

**2000-02909 Virus Isolation and Experimental Transmission of Bison MCF Gammaherpesvirus**

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Strengthening Standard Award; Grant 2001-35204-10151; $180,000; 2 Years

Malignant catarrhal fever (MCF) recently emerged as an important economic constraint on the rapidly growing commercial bison industry. The disease is caused by a virus of sheep, ovine herpesvirus-2 (OHV-2), which is widespread in American flocks. Control of MCF is hindered by several factors, chiefly an inability to isolate the causative virus in the laboratory, and our limited understanding of how the virus is transmitted naturally. Isolation of the virus is the first step in conducting experimental studies that will generate definitive data about transmission of the disease to susceptible animals, as well as incubation periods and immunity. This study will be done in two stages. In the first year we will isolate OHV-2 from selected lambs that shed large amounts of virus, as determined by a quantitative PCR technique developed

in our laboratory. Tissues will be collected from candidate high-shedder lambs in the US Sheep Experiment Station in Idaho. We will also attempt to isolate the virus from tissues of bison with naturally acquired MCF. Candidate OHV-2 isolates will be inoculated into OHV-2-negative sheep and bison in the second year. Inoculated sheep will be monitored for evidence of infection, such as seroconversion and appearance of viral DNA in PBL. Inoculated bison will be monitored for evidence of infection and clinical disease consistent with MCF, to establish that OHV-2 is indeed the sole agent responsible for the disease. Successful isolation of the virus and demonstration that it causes MCF are critical for the development of rapid, accurate diagnostic tests and effective control measures in bison and cattle.